

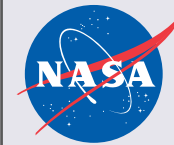


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Development of a metagenomic approach for ultra-low biomass samples from spacecraft surfaces

Lisa Guan and Parag Vaishampayan
Biotechnology and Planetary Protection Group

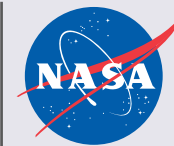
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As NASA missions evolve:

- Face more demanding scientific objectives
 - Assembly and verification of an ultraclean spacecraft
 - Highly sensitive instruments
- Need more sensitive assays for the detection of microbial bioburden on spacecraft hardware
- Require exploration of new methods and techniques for biological contamination and science

Mars 2020 Genetic Inventory Task

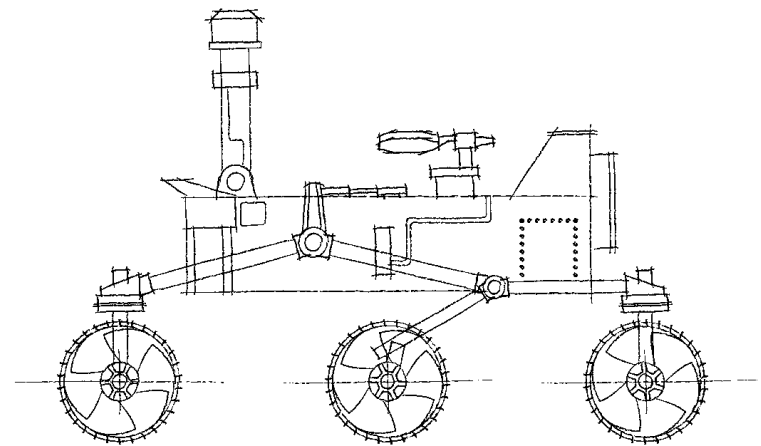


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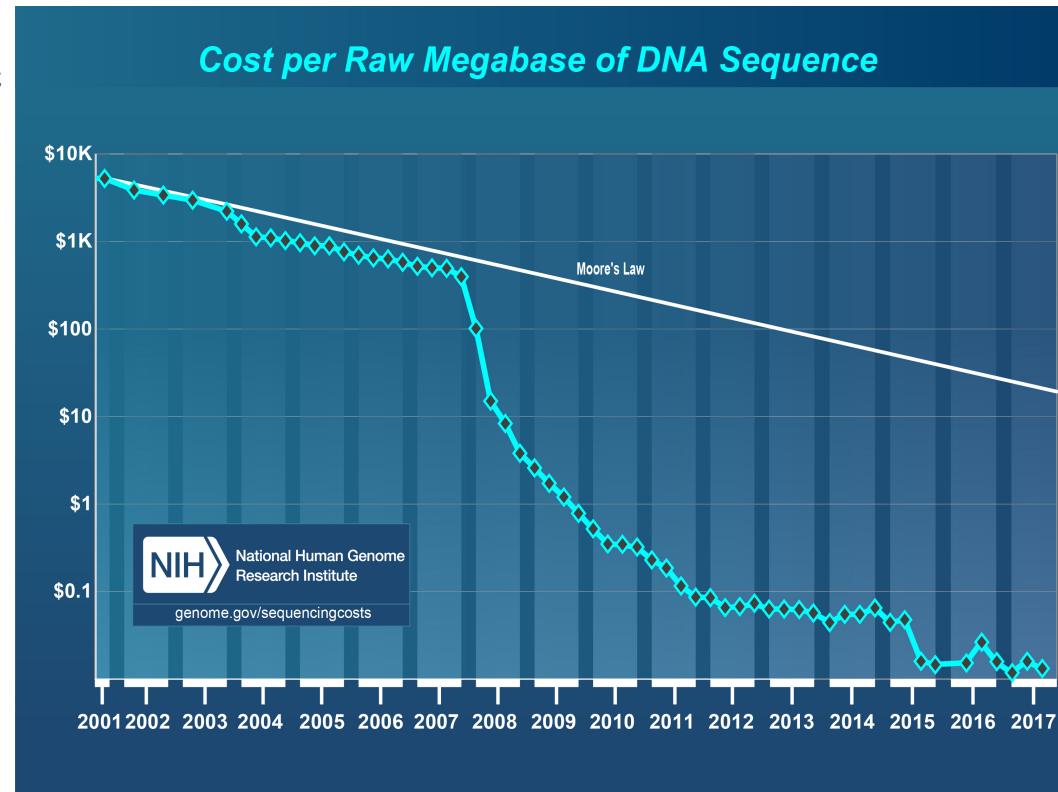
The project shall identify, quantify, document, and archive potential pre-launch terrestrial contamination sources, both organic compounds and organisms, and provide mechanisms to support characterization of round-trip terrestrial contamination.

- For Genetic Inventory (GI), we'll be using metagenomics, a molecular biology technique commonly used in the biomedical industry
- Metagenomics allows us to comprehensively capture microbial genetic information associated with spacecraft surfaces.
- With it, we'll be able to identify potential contamination sources and quantify their relative abundances.



Mars 2020 Project

- Culture-independent, direct genetic analysis by sequencing DNA from microbial communities in samples
- Has become increasingly mainstream due to high-profile projects such as the Human Microbiome Project (2012)
- Has become more accessible due to the decrease in sequencing costs





- Methods include:
 - Shotgun metagenomics
 - **who** is present in an environmental community
 - **what** their functions are
 - Does not use amplification of DNA
 - Targeted gene metagenomics
 - **who** is present in an environmental community
 - Uses PCR amplification of conserved marker genes
 - Target taxonomic groups (bacteria, fungi, etc.)

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Previous work – Microbial Diversity of Cleanrooms



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APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2009, p. 6559–6567
0099-2240/09/\$08.00+0 doi:10.1128/AEM.01073-09

Vol. 75, No. 20

Comprehensive Census of Bacteria in Clean Rooms by Using DNA Microarray and Cloning Methods^{¶†}

Myron T. La Duc,^{1,‡} Shariff Osman,^{2,§} Parag Vaishampayan,¹ Yvette Piceno,² Gary Andersen,¹ J. A. Spry,¹ and Kasthuri Venkateswaran^{1*}

ASTROBIOLOGY
Volume 10, Number 5, 2010
© Mary Ann Liebert, Inc.
DOI: 10.1089/ast.2009.0443

High-Density 16S Microarray and Clone Library–Based Microbial Community Composition of the Phoenix Spacecraft Assembly Clean Room

Parag Vaishampayan,¹ Shariff Osman,² Gary Andersen,² and Kasthuri Venkateswaran^{1*}



Pyrosequencing-Derived Bacterial, Archaeal, and Fungal Diversity of Spacecraft Hardware Destined for Mars

Myron T. La Duc,^a Parag Vaishampayan,^a Henrik R. Nilsson,^b Tamas Torok,^c and Kasthuri Venkateswaran^a

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2010, p. 2837–2845

0099-2240/10/\$12.00 doi:10.1128/AEM.02167-09

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Vol. 76, No. 9

Diversity of Anaerobic Microbes in Spacecraft Assembly Clean Rooms^{¶†}

Alexander Probst,^{1,‡} Parag Vaishampayan,¹ Shariff Osman,² Christine Moissl-Eichinger,³ Gary L. Andersen,² and Kasthuri Venkateswaran^{1*}

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 2011, p. 5438–5444

0099-2240/11/\$12.00 doi:10.1128/AEM.00192-11

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Comparison of Innovative Molecular Approaches and Standard Spore Assays for Assessment of Surface Cleanliness[¶]

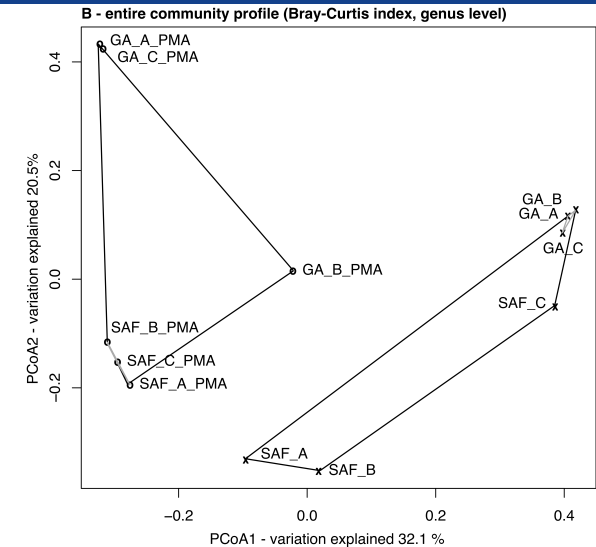
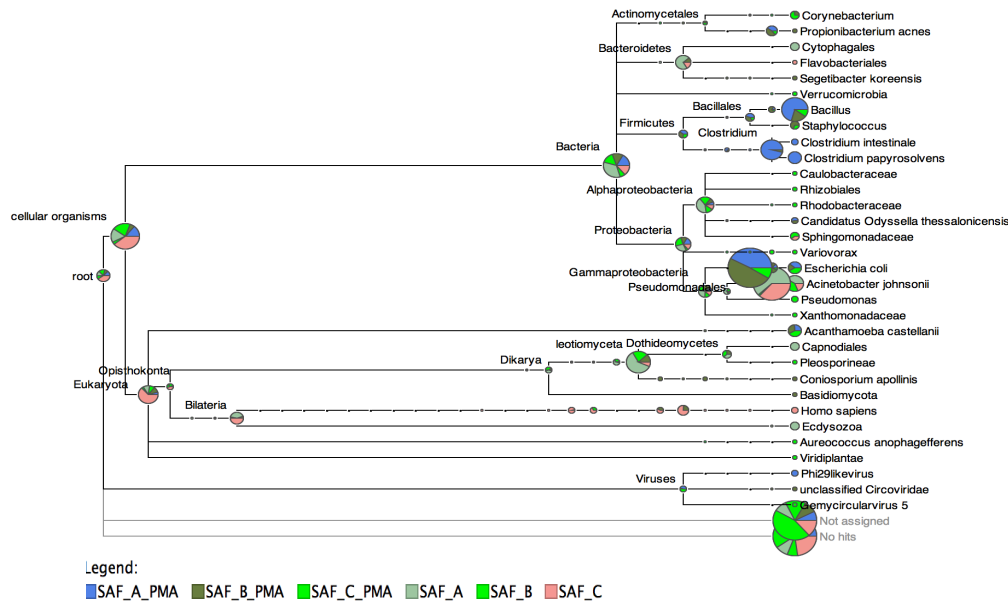
Moogega Cooper,¹ Myron T. La Duc,¹ Alexander Probst,¹ Parag Vaishampayan,¹ Christina Stam,¹ James N. Benardini,¹ Yvette M. Piceno,² Gary L. Andersen,² and Kasthuri Venkateswaran^{1*}

Metagenome Study Overview



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The spacecraft assembly facility exhibits a significantly different community composition than the gowning area.

Weinmaier et al. *Microbiome* (2015) 3:62
DOI 10.1186/s40168-015-0129-y



Microbiome

RESEARCH

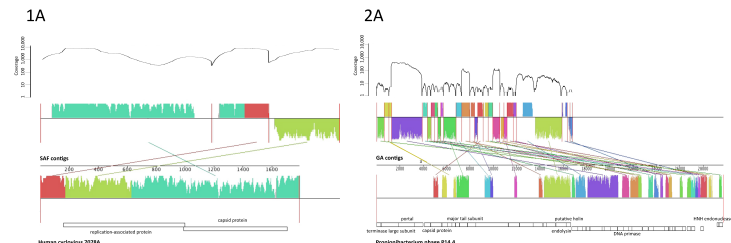
Open Access



CrossMark

A viability-linked metagenomic analysis of cleanroom environments: eukarya, prokaryotes, and viruses

Thomas Weinmaier^{1†}, Alexander J. Probst^{2†}, Myron T. La Duc^{3,4}, Doina Ciobanu⁵, Jan-Fang Cheng⁵, Natalia Ivanova⁵, Thomas Rattei¹ and Parag Vaishampayan^{3*}



Genome reconstruction provides evidence for the presence of human cyclovirus 7078A and Propionibacterium phage P14.4 in the cleanroom environment.

Mars 2020 Project-8

Sample Collection

Method: Swabs or Wipes

Hardware



Facility



Samples

Sample Processing

DNA Extraction

- Samples pooled prior to extraction
- Automated platform
- Purify DNA
- DNA pooling if necessary

Genomic Library Prep/Sequencing

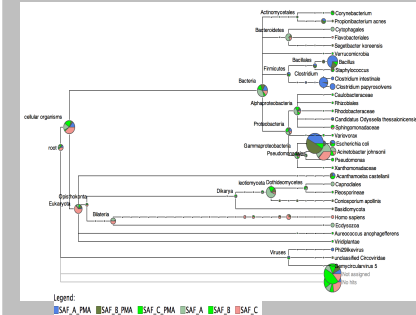
- >10 pg DNA: Whole-genome metagenome
- <10 pg DNA: Amplicon based sequencing

Data Analysis

- Bioinformatic analysis
- Passenger List

Pooled DNA

Raw Data

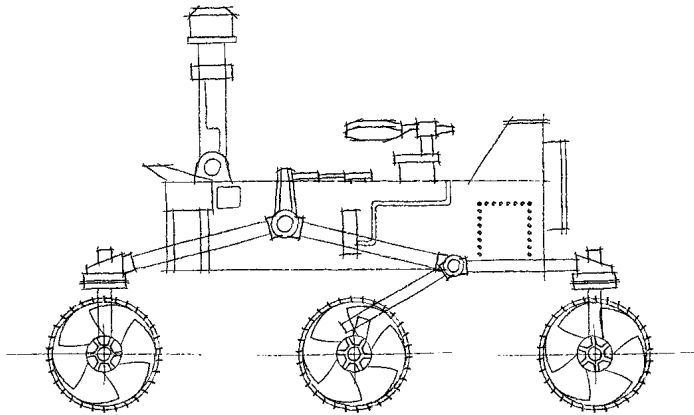


Expected DNA quantities



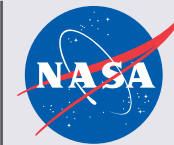
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	Expected DNA yield from hardware
Per Swab	0.25 pg
Per Wipe	90 pg

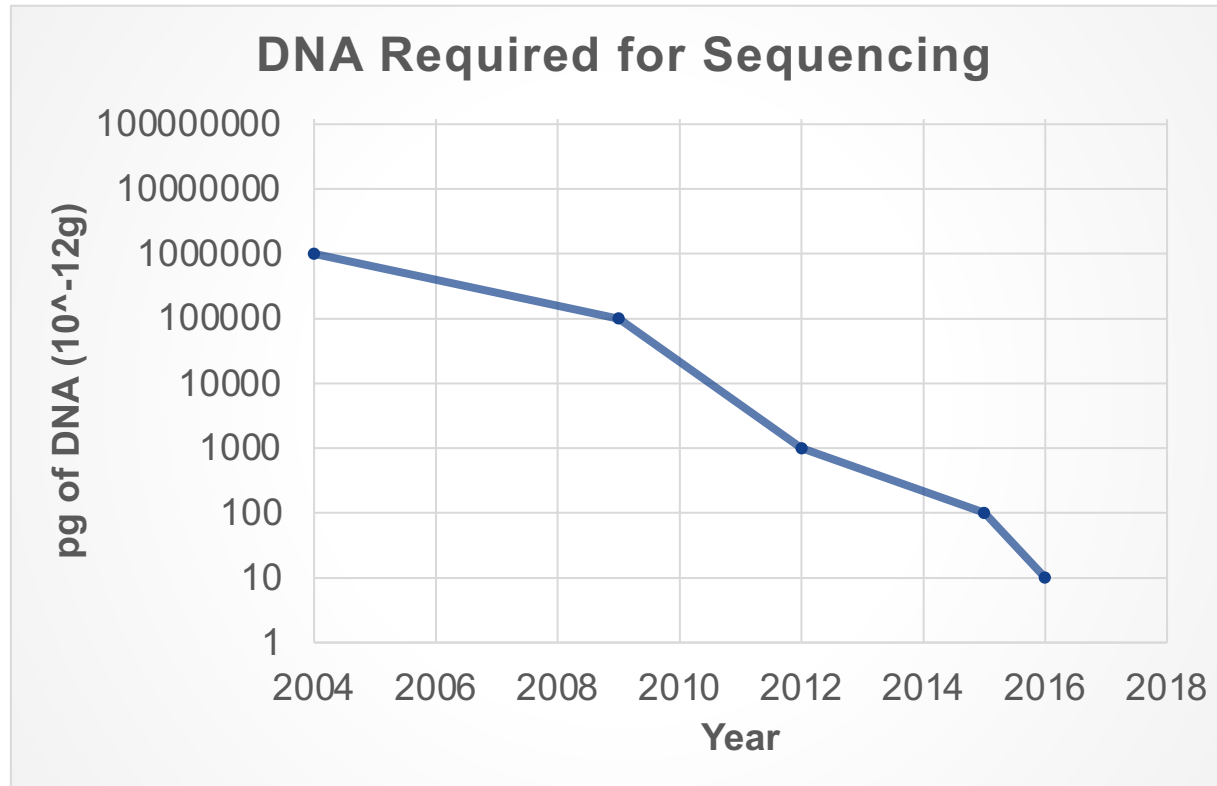
Currently, the greatest challenge is to yield enough DNA for Next-Generation Sequencing.



- Our lowest limit of detection is 1 pg DNA
- Library construction is possible at 10 pg DNA
 - Lower limit of DNA extraction
- Unbiased library construction requires at least 100 pg of DNA

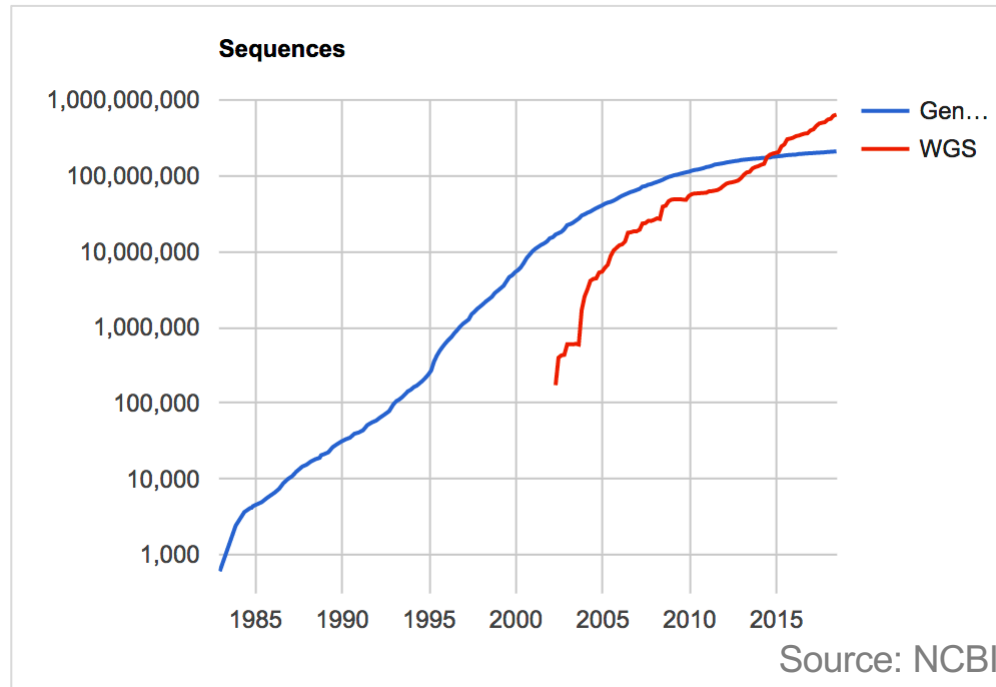
- Due to our current limitations from low biomass, we will be archiving samples throughout our workflow for analysis with future technologies.
- Samples will be stored long-term in a Genetic Inventory Archive at -80°C .
- Due to the rapidly advancing nature of genomic analysis technologies, we expect to see significantly improved methods and instruments in the coming years.





Sources: Oulas et al. 2015, Syed et al. 2009, Parkinson et al. 2012, Bowers et al. 2015, Rinke et al. 2016

The amount of DNA required for library construction for sequencing has decreased by approximately an order of magnitude every year.



As more people are sequencing and sharing data in public databases we will have more references to compare and analyze our samples with.

Currently, the number of bases submitted to NCBI's GenBank doubles every 18 months.

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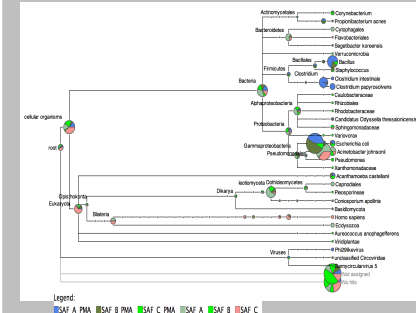
Archive

Pooled DNA

Archive

Raw Data

Archive





- Apply functional gene analysis to capture the functional traits of microorganisms present on in cleanrooms and spacecraft hardware
- Can identify presence of genes associated with resistance to harsh environments (i.e. heat resistance, radiation resistance, etc.)
- Metagenomics could also be applied to future missions such as:
 - Potential Mars Sample Return
 - Europa Clipper
 - Europa Lander Concept

Acknowledgements



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